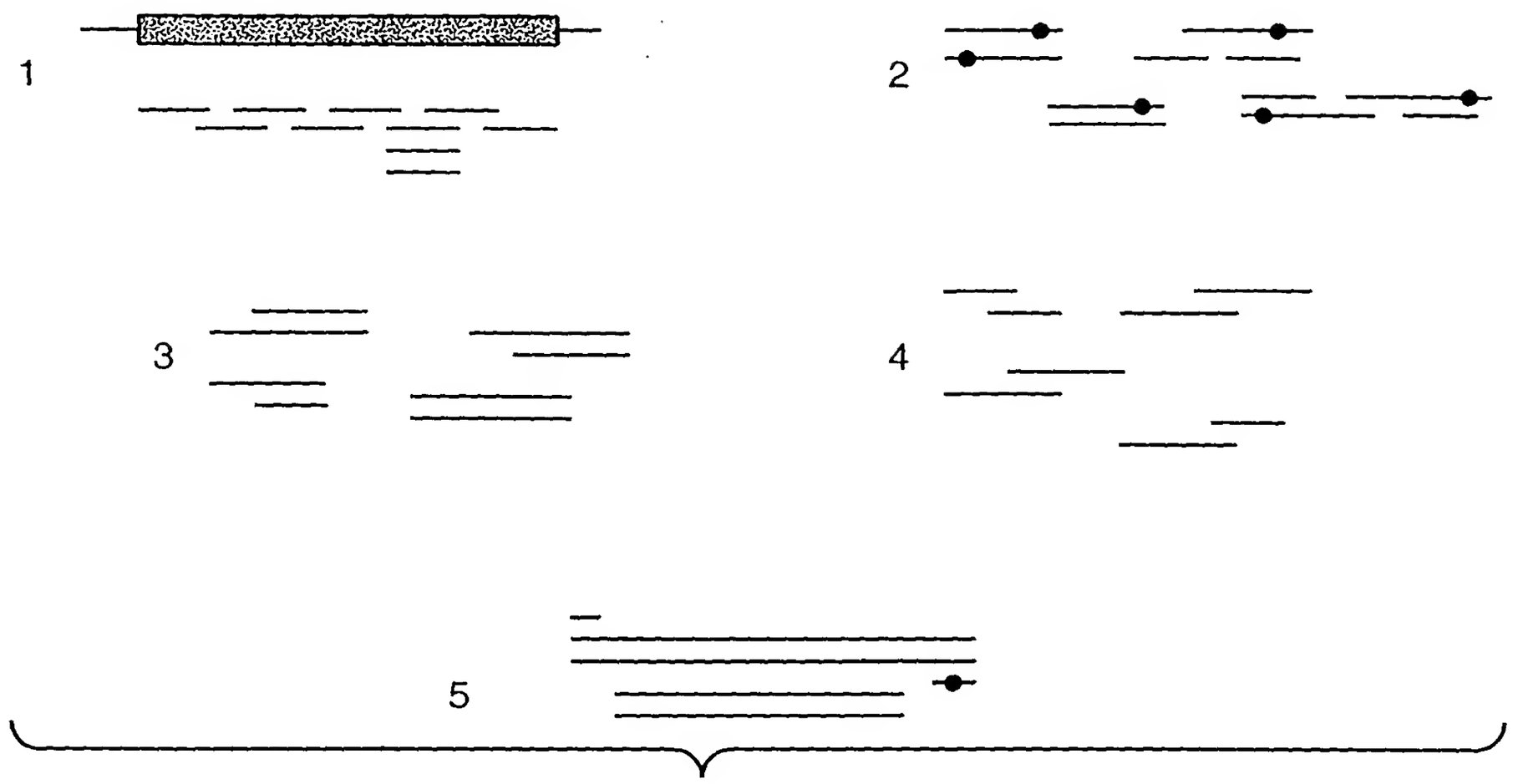


FIG._1

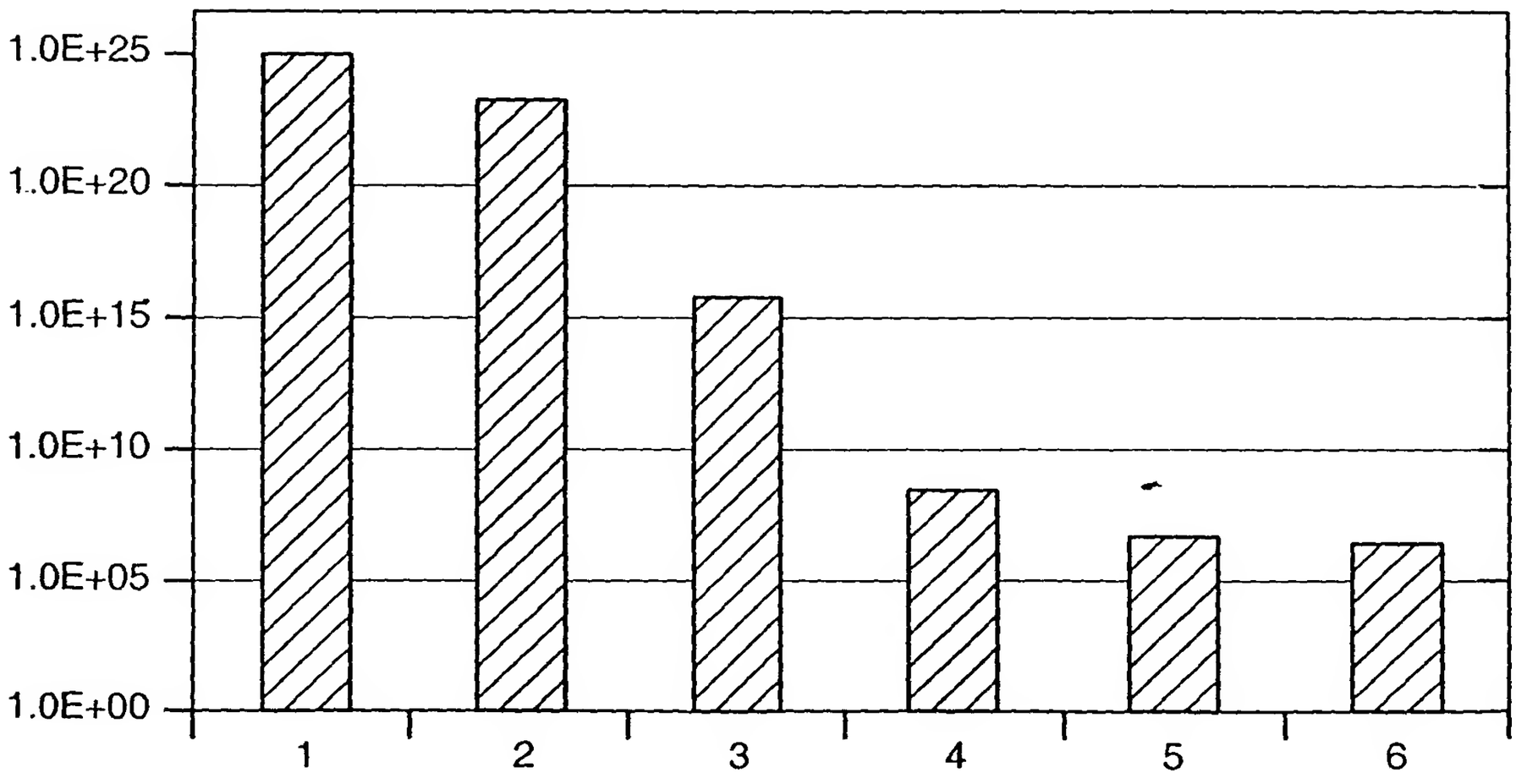


FIG._2

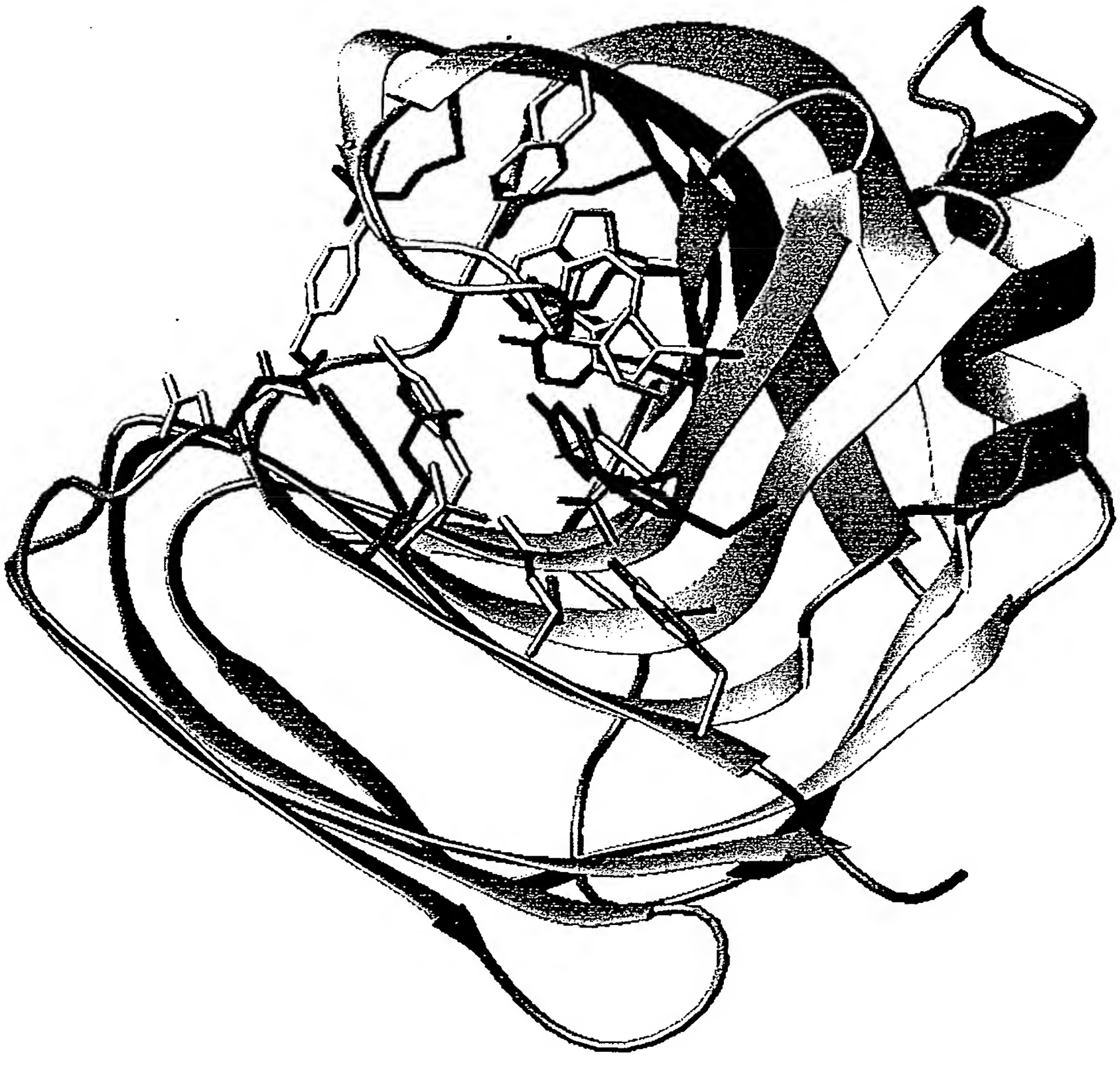


FIG._3

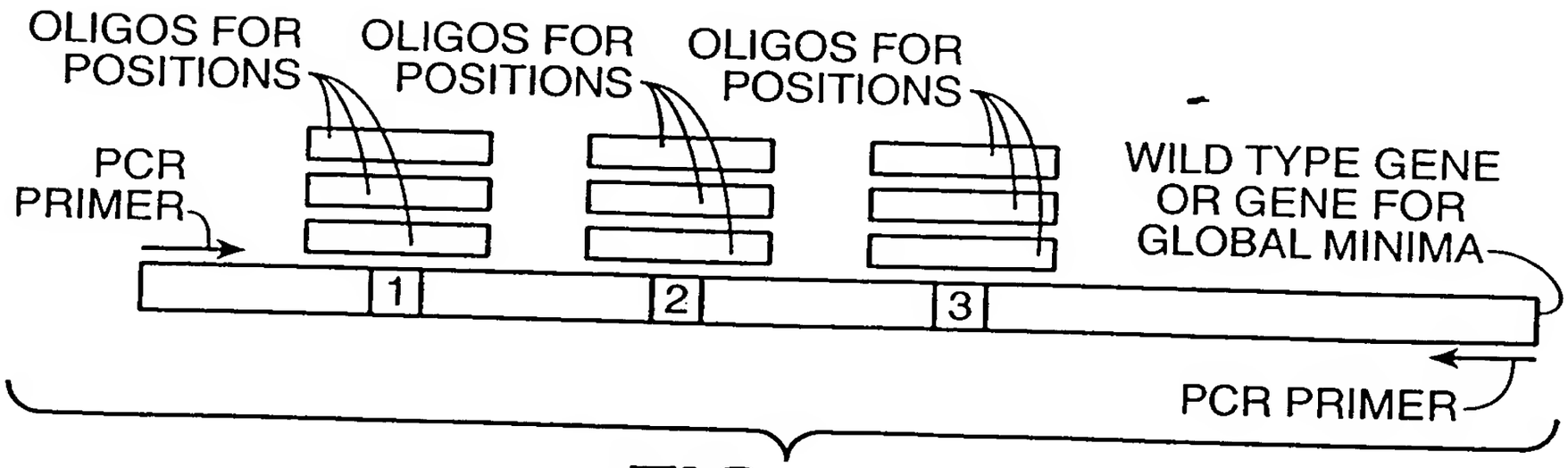

OLIGOS FOR POSITIONS
OLIGOS FOR POSITIONS
OLIGOS FOR POSITIONS
PCR PRIMER
WILD TYPE GENE OR GENE FOR GLOBAL MINIMA
1
2
3
PCR PRIMER

FIG._5

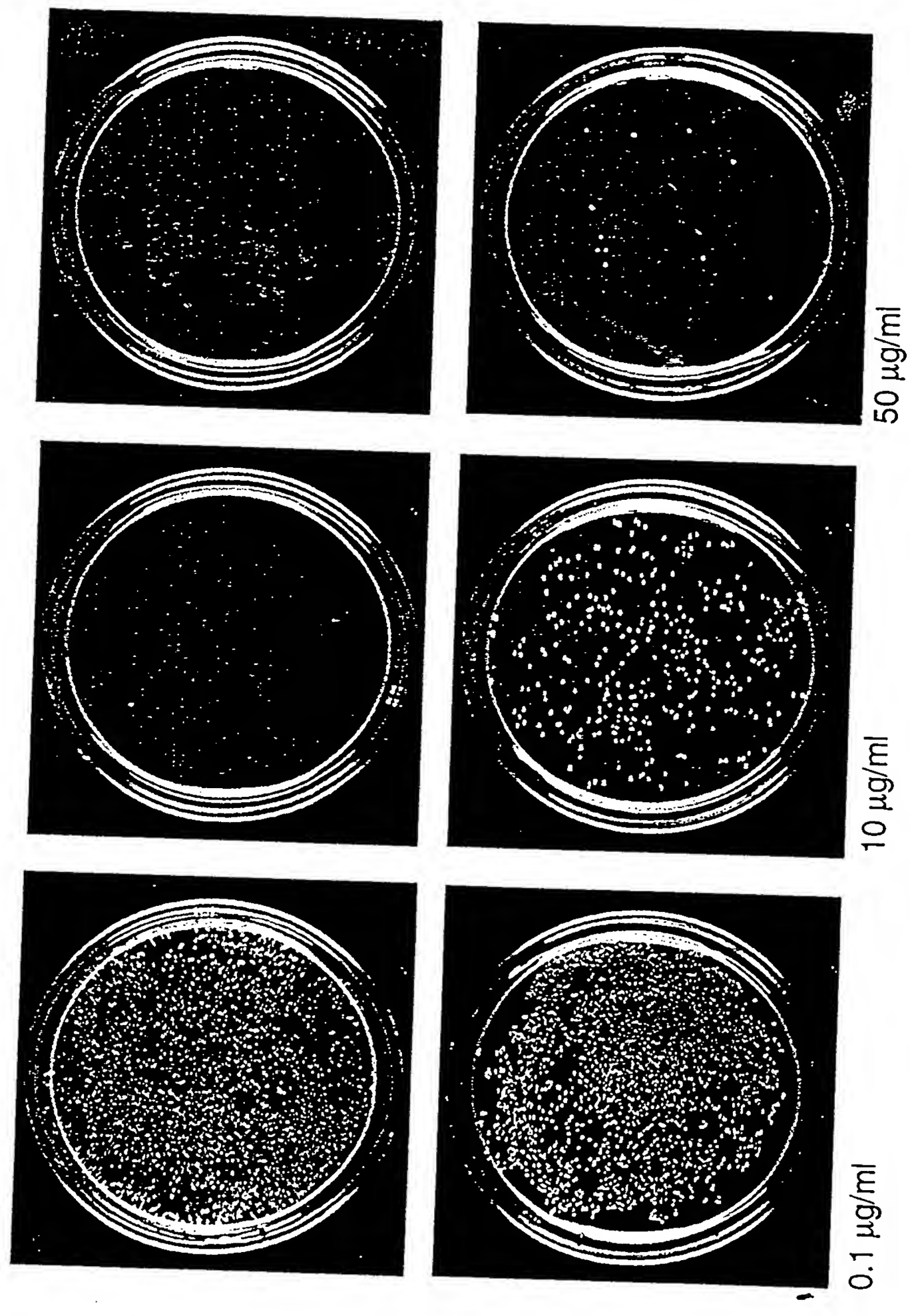
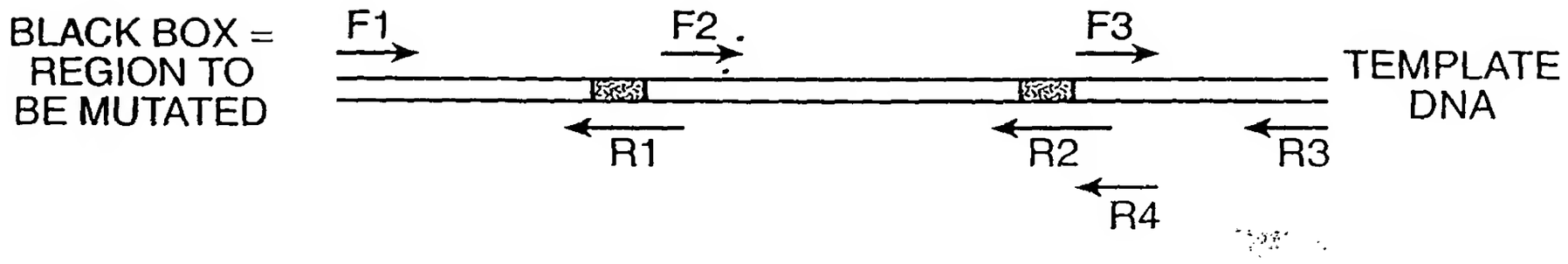


FIG._4



STEP 1: SET UP 3 PCR REACTIONS:

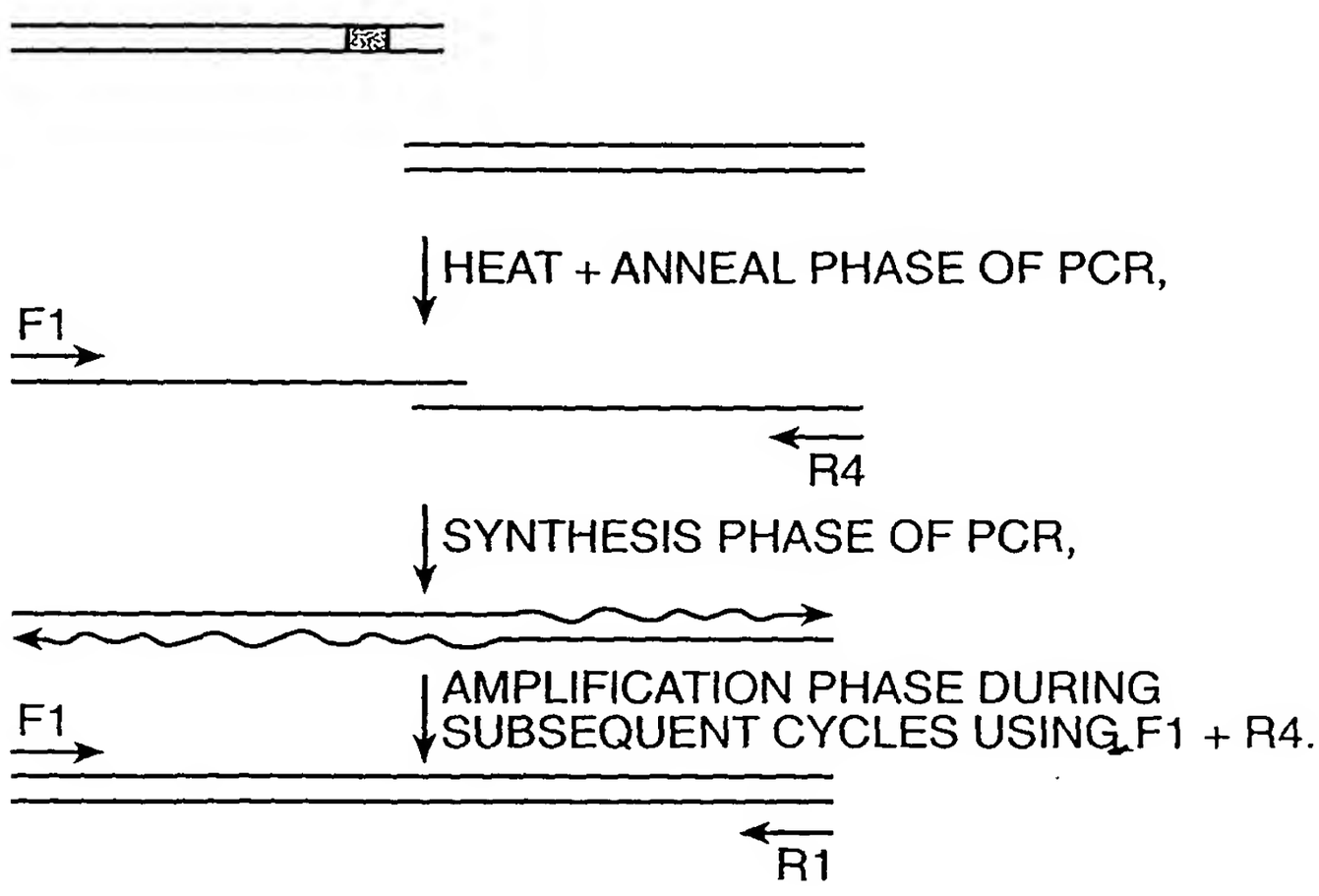
PRODUCTS:

TUBE 1:

TUBE 2:

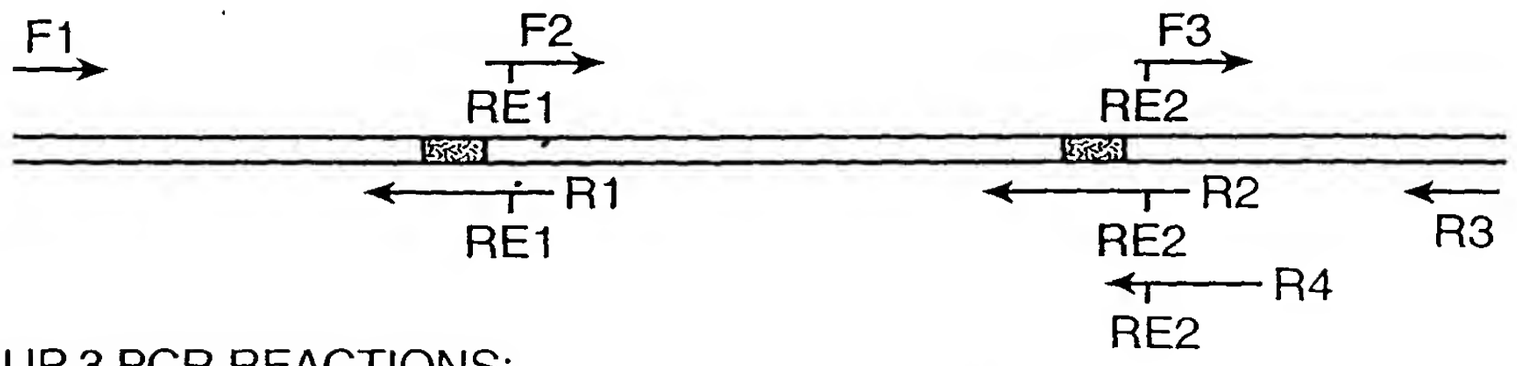
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



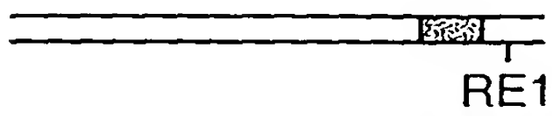
STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG._6

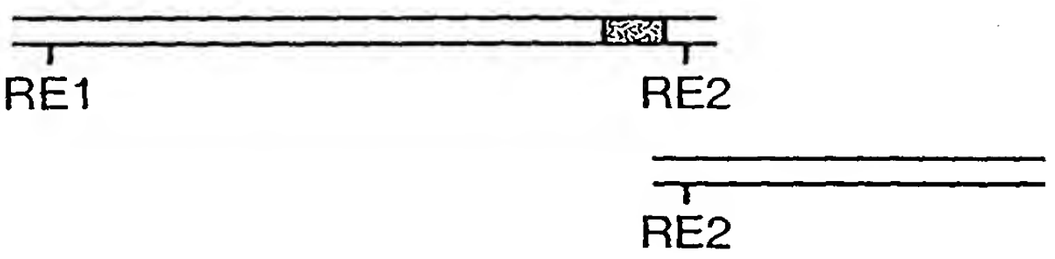


STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:



TUBE 2:



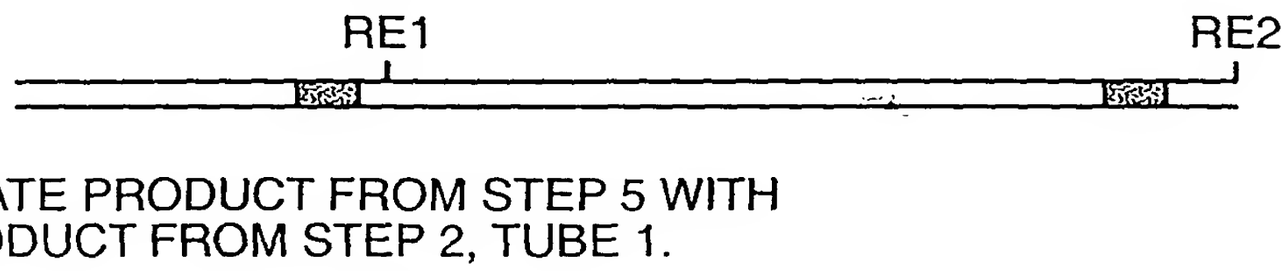
TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.

STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.

STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.

STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG._7

DIAGRAM 3

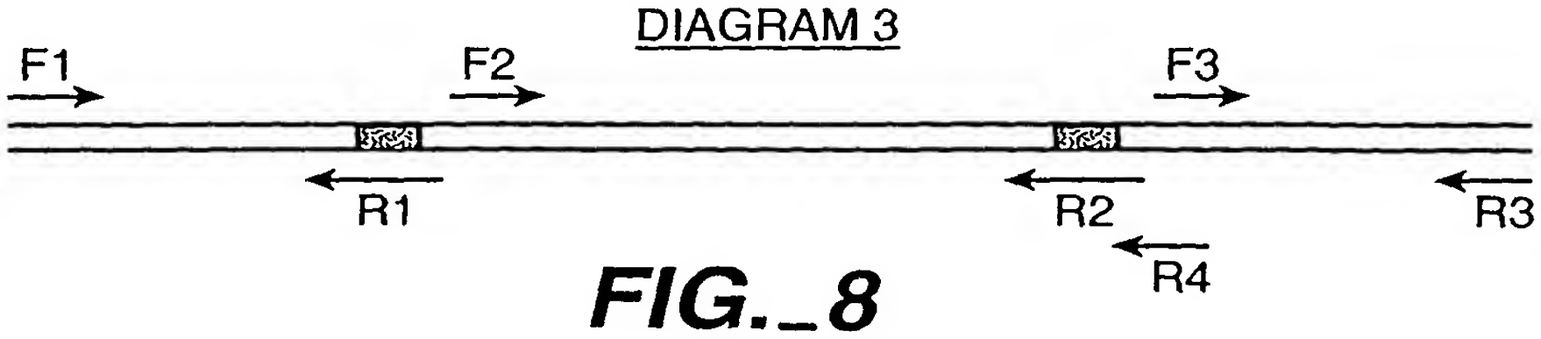


FIG._8

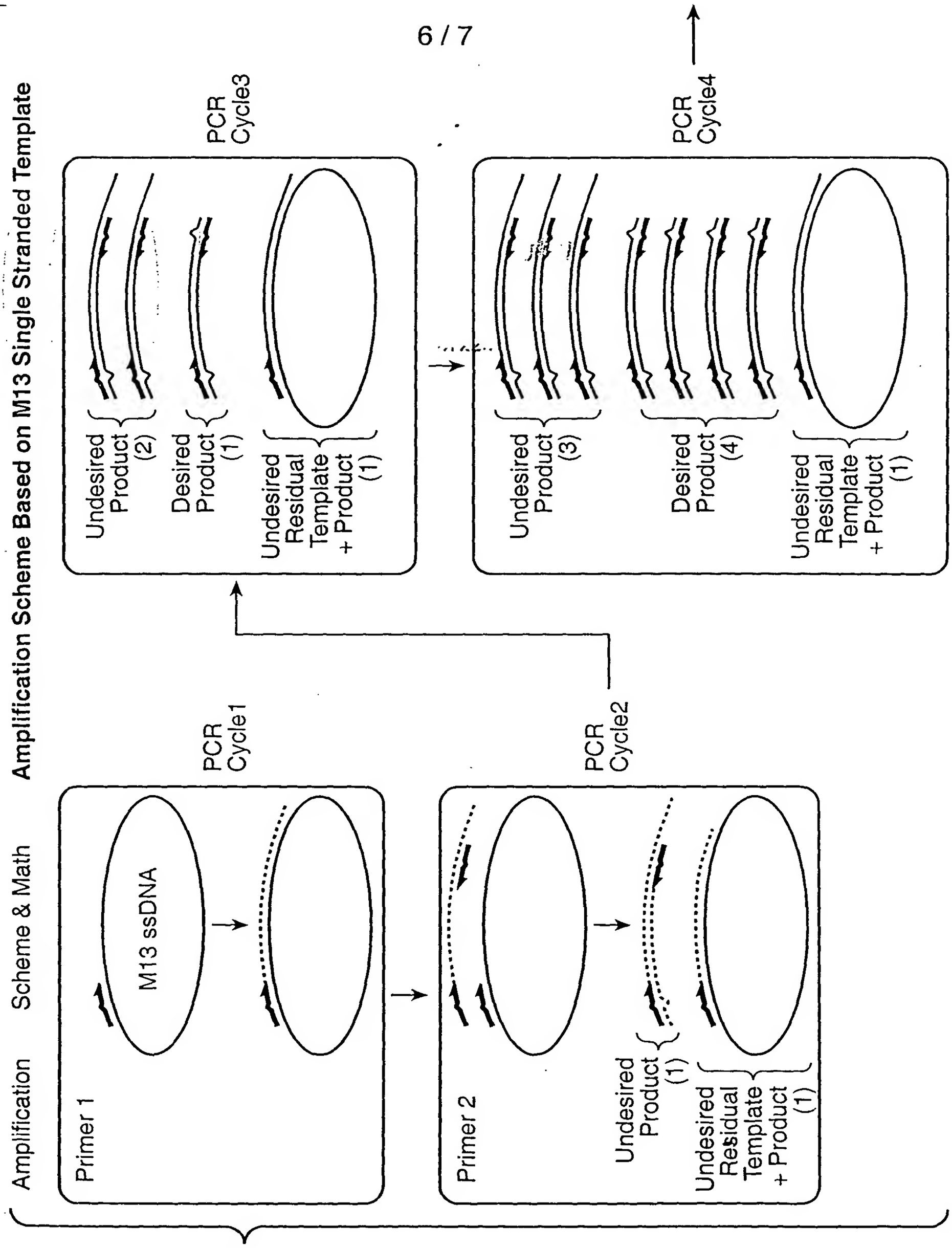
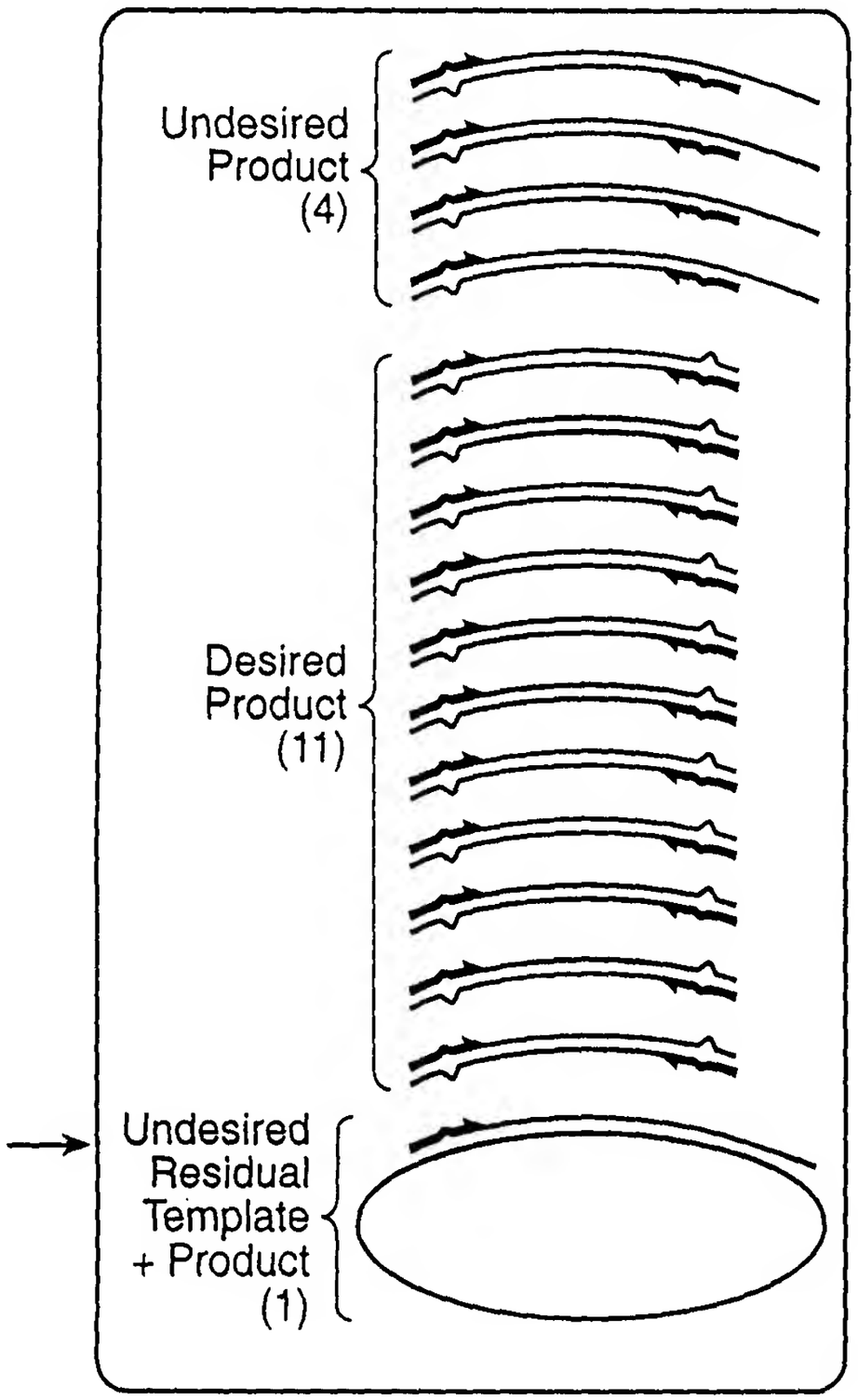

Amplification Scheme & Math
Amplification Scheme Based on M13 Single Stranded Template
Primer 1
M13 ssDNA
Primer 2
PCR Cycle1
PCR Cycle2
PCR Cycle3
PCR Cycle4
Undesired Product (1)
Undesired Residual Template + Product (1)
Undesired Product (2)
Desired Product (1)
Undesired Residual Template + Product (1)
Undesired Product (3)
Desired Product (4)
Undesired Residual Template + Product (1)

FIG._9A

Amplification Scheme Based on M13 Single Stranded Template



Numerical Progression of Desired Product with Increasing PCR Cycles

PCR Cycles	Desired Product	Undesired Products and Residual Template	Percent Desired Product in Total Product
1		1	
2	0	2	0.00%
3	1	3	25.00%
4	4	4	50.00%
5	11	5	68.75%
6	26	6	81.25%
7	57	7	89.06%
8	120	8	93.75%
9	247	9	96.48%
10	502	10	98.05%
11	1013	11	98.93%
12	2036	12	99.41%
13	4083	13	99.68%
14	8178	14	99.83%
15	16369	15	99.91%
16	32752	16	99.95%
17	65519	17	99.97%
18	131054	18	99.99%
19	262125	19	99.99%
20	524268	20	100.00%

FIG._9B